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(54) Title: CLONING OF LFA-1

(57) Abstract

The invention features substantially pure recombinant β-subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, an analog thereof, or a fragment thereof composed of at least 10% of a contiguous sequence of the β-subunit; a cDNA sequence coding therefor; and a vector containing a DNA sequence coding therefor. The invention also features monoclonal antibodies raised against the recombinant β-subunit of human LFA-1. Methods of using the glycoprotein and analogs thereof and antibodies are also disclosed as is a nucleic acid molecular hybridization assay using DNA probes.

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CLONING OF LFA-1

Background of the Invention

The work described herein was performed with the aid of government funding, and the government therefore has certain rights in the invention. Specifically, the work was supported by N.I.H. grants CA 31798 and AI 05877.

This invention relates to cellular adhesion.

Cellular adhesion is a critical function for guiding migration and localization of cells, and for maintaining the integrity of the body. Receptors for extracellular matrix components such as fibronectin, laminin, and vitronectin mediate cellular adhesion during morphogenesis and wound healing. In the immune system, regulatory networks require intimate cell-cell interaction among lymphocytes and antigen-presenting accessory cells, and cell-mediated cytolysis involves direct contact between the effector cell and virally-infected or transformed target cells. Leukocyte-endothelial interactions are important in leukocyte mobilization into inflammatory sites and in lymphocyte recirculation. These cellular adhesion reactions are mediated in part by a family of structurally related glycoproteins, LFA-1, Mac-1, and p150,95, all of which share a common ß-subunit (hereinafter referred to as the ß-subunit of human LFA-1). (Springer et al., 314 Nature 540, 1985; Springer et al., Ann. Rev. Immunol. Vol. 5, 1987; both hereby incorporated by reference).

Summary of the Invention

In general, the invention features a) substantially pure recombinant ß-subunit of a human glycoprotein concerned with cellular adhesion, or b) a biologically active fraction of this ß-subunit, c) an

analog of the ß-subunit, or d) a fragment of the ß-subunit, composed of at least 10% of a contiguous sequence of the ß-subunit. The invention also features a cDNA sequence encoding the ß-subunit; and a vector containing said cDNA sequence. By recombinant subunit is meant the polypeptide product of recombinant DNA encoding the ß-subunit, i.e., the polypeptide expressed from DNA which is not in its naturally occuring location within a chromosome. By natural subunit is meant that subunit produced naturally in vivo from naturally occuring and located DNA. By analog is meant a polypeptide differing from the normal polypeptide by one or more amino acids, but having substantially the biological activity of the normal polypeptide. The invention also features any monoclonal antibody (MAb) raised against the recombinant ß-subunit, a biologically active fraction, an analog, or a fragment thereof composed of at least 10%, preferably at least 80%, of a contiguous sequence of the ß-subunit of a human glycoprotein.

The cDNA sequence encoding the LFA-1 ß-subunit or a fragment thereof may be derived from any of the naturally occuring genes encoding it, or synthesized chemically. Variations in this sequence which do not alter the amino acid sequence of the resulting protein, or which do not significantly alter the biological activity of the protein, are also acceptable, and are within this invention.

Preferably the human glycoprotein is LFA-1, Mac-1 or p150,95.

As will be described in more detail below, the invention permits the diagnosis and treatment of a variety of human disease states.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

Description of the Preferred Embodiments

The drawings are first briefly described.

Drawings

Fig. 1 is the DNA coding sequence of the ß-subunit of LFA-1, Mac-1 and p150,95. Potential N-glycosylation sites are marked with triangles.

Fig. 2 is a comparison of the amino acid sequence predicted from the cDNA in Fig. 1, and the amino acid sequence derived from enzyme digests of the ß-subunit of LFA-1. Ambiguous determinations of amino acids are bracketed. The code for amino acids is as follows:

Ala,	A	-alanine
Arg,	R	-arginine
Asn,	N	-asparagine
Asp,	D	-aspartic acid
Cys,	C	-cysteine
Gln,	Q	-glutamine
Glu,	E	-glutamic acid
Gly,	G	-glycine
His,	H	-histidine
Ile,	I	-isoleucine
Leu,	L	-leucine
Lys,	K	-lysine
Met,	M	-methionine (start)
Phe,	F	-phenylalanine
Pro,	P	-proline
Ser,	S	-serine
Thr,	T	-threonine

Trp,	W	-tryptophan
Tyr,	Y	-tryosine
Val,	V	-valine

Methods

In general, the ß-subunit of any of the above described related glycoproteins is isolated by standard procedures and the amino acid sequence of at least a part of it determined. From this analysis a synthetic oligonucleotide probe, corresponding to the amino acid sequence, is synthesized and used as a probe for a genomic or cDNA library containing a DNA sequence encoding the ß-subunit. An example of this procedure is given below. One skilled in the art will realize that this represents only one of many methods which can be used to achieve cloning of the gene encoding the LFA-1 ß-subunit.

Purification of the ß-Subunit

MAb's directed against the alpha subunits of p150,95, Mac-1, and LFA-1, were used to affinity purify their respective proteins from three different sources. The p150,95 protein was purified from hairy cell leukemia spleens (Miller et al., 1986, 137 J. Immunol. 2891, hereby incorporated by reference); Mac-1 was purified from pooled human leukocytes (Miller et al., supra); and LFA-1 was purified from the SKW3 T cell line using TS1/22 monoclonal antibody (Sanchez-Madrid et al. 1983, J. Exp. Med. 158:586, hereby incorporated by reference).

Preparative SDS-PAGE gels were run using the method of Laemmli (Hunkapiller et al., 1983, Meth. Enzym. 91:227). 0.1 mM Na thioglycolate was added to the upper chamber to reduce the level of free radicals in the gel. Bands were visualized by soaking the gel

for several minutes in 1 M KCl and then excised. The ß-subunit was electroeluted using the apparatus and method described by Hunkapillar et al., supra. The purified protein was reduced with 2 mM DTT in the presence of 2% SDS and alykylated with 5 mM iodoacetic acid in the dark. (In some cases, the protein was reduced and alkylated prior to running the preparative gel.)

Amino acid sequencing

The above samples were precipitated using four volumes of ethanol at -20°C for 16 hr, and the protein pellet redissolved in 30-50 ml of 0.1 M NH_4CO_3 containing 0.1 mM $CaCl_2$ and 0.1% Zwittergent 3-14 (Calbiochem, San Diego, CA). The sample was then digested with 1% w/w trypsin for 6 hr at 37°C. At 2 and 4 hr during the incubation, additional trypsin (1% w/w) was added.

The tryptic peptides were resolved by reverse phase HPLC (Beckman Instruments) with a 0.4 X 15 cm C4 column (Vydac, Hesperig, CA), and eluted from a 2 hr linear gradient from 0 to 60% acetonitrile. 0.1% TFA was included in both the aqueous and organic solvents. The peaks were monitored at 214 and 280 nm and collected into 1.5 ml polypropylene tubes. The fractions were concentrated to 30 ml or less on a speed-vac apparatus, and selected peptides subjected to sequence analysis using a gas phase microsequenator (Applied Biosystems, Foster City, CA).

Example: ß-subunit of p150,95

p150,95 was affinity purified from the spleens of human patients with hairy cell leukemia using a monoclonal antibody specific for the alpha subunit (MW approx. 150,000, Miller et al., supra). Analysis of the

purified protein by SDS-PAGE and silver staining revealed the characteristic alpha and beta subunit, with no significant amounts of contaminating proteins. The ß-subunit band was excised from a preparative SDS-PAGE gel and electroeluted, as described above.

The N-terminus of the beta subunit was blocked and therefore could not be sequenced. Internal amino acid sequence information was obtained by digesting the ß-subunit with trypsin. The tryptic peptides were resolved by reverse phase HPLC and eluted on a 60% acetonitrile gradient. Peaks analyzed by absorbance at 214 and 280 nm were collected and applied to a gas phase microsequenator.

The peptide sequences of two of these fragments is:

P-61 Peptide Sequence:

LeuTyrGluAsnAsnIleGlnProIlePheAlaValThrSer

P-20 Peptide Sequence:

ThrAspThrGlyTyrIleGlyLys.

Two strategies were adopted for constructing oligonucleotide probes. A unique sequence 39mer was designed from peptide P-61 based on human codon usage frequency (Lathe, 1985, J. Mol. Biol. 183:1). Its sequence is:

3'- GACATACTCTTGTTGTAGGTCGGGTAGAAACGACACTGG -5'.

In addition, two sets of mixed sequence probes were constructed such that every possible sequence was represented. A 20mer of 96-fold redundancy was derived from peptide P-61, and a 17mer of 192-fold redundancy was constructed based on the sequence from a different peptide fragment of the ß-subunit, P-20. These sequences are given below.

20mer, Mixed Sequence 3'- ATACTATTATTATAAGTCCC -5'
G C G G C T
G

17mer, Mixed Sequence 3'- CTATGACCAATATAACC -5'
G C C G G
G G T
T T

The 39mer and the mixed sequence 20mer were used to probe a Northern blot of poly A+selected RNA from PMA-activated U937 cells. The U937 cells, JY lymphoblastoid cells, HeLa cells, and CO3 cells (Springer et al., 1984, J. Exp. Med. 160:1901, an EBV-transformed cell line from a healthy donor) were grown in RPMI 1640 containing 10-15% fetal calf serum in a humidified atmosphere of 5% CO_2 at 37°C. The U937 cells were activated with 2 ng/ml PMA for three days prior to harvesting. The cells were lysed in a 4M guanidinium isothiocyanate solution, and RNA isolated in a 5.7M CsCl gradient. Poly A+ mRNA was selected with oligo (dT)-cellulose columns (Maniatis et al., Molecular Cloning: A laboratory manual, Cold Spring Harbor Laboratory, N.Y., 1982) or oligo (dT)-affinity paper (Amersham). This RNA was denatured and sized on a 1% agarose gel containing formaldehyde (Maniatis et al., supra) and transferred to nylon membranes (BioRad) in 20X SSC. A lane containing 28S and 18S ribosomal RNA from human cells or 23S and 16S rDNA from Escherichia coli was run to provide molecular weight standards.

The filters were hybridized with nick-translated probe DNA at 42°C for 18 hr in 5 X SSPE, 50% formamide, 10% dextran sulfate, 1 X Denhardts, 0.5% SDS and 100 ug/ml denatured salmon sperm DNA, and washed at high stringency (65°C) in 0.2 X SSC and 0.1% SDS. Both probes identified a band of approximately 3 kb.

The 39mer gave a much stronger signal and was chosen for the primary screening of a cDNA library.

A human tonsil cDNA library (gift of L. Klickstein) was size-selected for inserts of 2kb or greater and constructed in ggt11 (Wong et al., 1985, Proc. Nat. Acad. Sci. U.S.A. 82:7711). The original library of 4 X 10^6 recombinants was amplified once, and 200,000 recombinants plated at a density of 7500 plaques/100mm plate. The plaques were amplified in situ on duplicate nitrocellulose filters, as described by Woo (1979, Meth. Enzym. 68:389).

The oligonucleotide probes were labeled with ^{32}P-ATP using polynucleotide kinase. The filters were prehybridized for at least 2 hr at 42°C in 6 X SCC, 1 X Denhardts, 0.5% SDS, 0.05% phosphate buffer, and 100 mg/ml of salmon sperm DNA. Hybridization with the 39mer was overnight at 42°C in prehybridization solution containing 20 mg/ml tRNA. The filters were washed at 53°C to 55°C with 6 X SSC, 0.1% SDS, and 0.05% phosphate buffer. The damp filters were covered with plastic wrap and exposed to film with an intensifying screen. Phage that gave positive signals on duplicate filters were plaque purified and rescreened with the 39mer at a higher wash temperature (60°C) and with 20mer and 17mer mixed sequence probes. 15 positive clones were picked. Eight of the clones crossreacted with each other and gave positive signals with the 20mer mixed sequence probe and the independent 17mer mixed sequence probe. These clones were chosen for further analysis.

To confirm the identity of the cDNA clones, a 263 bp PstI/EcoRI restriction fragment which hybridized to the 39mer was subcloned into M13 vector and sequenced by the Sanger dideoxy chain termination method as follows. The amino acid sequence deduced from the DNA

sequence is identical in 13 of 14 positions to the peptide sequence from which the 39mer probe was derived, including one amino acid which was not included in the design of the oligonucleotide. Furthermore, the predicted amino acid sequence shows that this peptide is preceded by a lysine and followed by an arginine, as expected for a tryptic fragment. The one mismatch may be due to normal polymorphism. The unique sequence oligonucleotide was 87% homologous to the cDNA sequence, despite the one amino acid mismatch.

The cDNA clones were restriction mapped by single and double restriction digests and, after end-labeling, by partial restriction digests (Maniatis et al., supra). Compatible restriction fragments were subcloned directly into M13 cloning vectors. Other fragments were first blunt ended with Klenow, T4 polymerase, or Mung Bean nuclease (Maniatis et al., supra) and ligated into the HincII or SmaI site of the M13 polylinker. The nucleotide sequence of both strands was determined by the dideoxy chain termination method of Sanger et al. (1977, Proc. Nat. Acad. Sci. U.S.A. 74:5463) using ^{35}S-dATP.

The complete nucleotide sequence and deduced amino acid sequence of the ß-subunit gene in the longest clone, 18.1.1 (2.8 kb in length), is shown in Figure 1. The first ATG is at position 73, and the sequence surrounding the ATG is consistent with the consensus rules for an initiation codon (Kozak 1984, Nucl. Acid. Res. 12:857). This putative initiation codon is followed by an open reading frame of 2304 bp, which could encode a polypeptide of 769 amino acids (aa). The stop codon ATC is followed by a 3' untranslated region of 394 bp. The poly A tail was not found, although a

consensus polyadenylation signal (AATAAA) is located 9 bp from the 3' end.

The deduced amino acid sequence of the cDNA clones was compared to peptide sequence data from the beta subunit of Mac-1, LFA-1, and p150,95 (Fig. 2). In addition to the P61 and P-20 peptide sequences given above, one other peptide was sequenced from the beta subunit of p150,95. Tryptic peptides were also prepared and analyzed from the beta subunit of purified Mac-1 and LFA-1. Each peptide sequence is found within the deduced amino acid sequence (Figs. 1 and 2). Thus, it can be concluded that the cDNA encodes the ß-subunit of human LFA-1.

The cDNA clones hybridize to a single mRNA species of approximately 3.0 kb, which is the same message identified by the 39mer oligonucleotide. This message is present in PMA-activated U937 cells ($LFA\text{-}1^+$, $Mac\text{-}1^+$, $p150,95^+$), JY lymphoblastoid cells ($LFA\text{-}1^+$, $Mac\text{-}1^-$, $p150,95^-$), and EBV-transformed cells from a normal donor ($LFA\text{-}1^+$, $Mac\text{-}1^-$, $p150,95^-$), but is absent in HeLa cells ($LFA\text{-}1^-$, $Mac\text{-}1^-$, $p150, 95^-$). Although clone 18.1.1 lacks the poly A tail, it is close to the estimated size of the RNA message.

Within the deduced polypeptide are two regions of sufficient length and hydrophobicity that could span the membrane bilayer. The first domain, which begins with the putative initiation methionine and extends 22 amino acids, has the characteristics of a signal sequence. This putative signal sequence is followed by a charged glutamine, a residue which is often cyclized at the N-terminal position. This would be consistent with the N-terminal blockage of the ß-subunit, if the signal sequence is cleaved during processing.

Use

The cDNA encoding the ß-subunit of human LFA-1 can be used to produce recombinant ß-subunit in large amounts. For example, the beta-subunit-encoding cDNA can be excised from the ggtl1 clones and introduced into an expression vector (plasmid, cosmid, phage or other type) to express the ß-subunit in E. coli, using standard techniques. Alternatively the clones may be inserted into other vectors, such as mammalian, insect, or yeast expression vectors, and used to produce recombinant ß-subunit in mammalian or yeast cells.

The subunits produced by the above methods can be readily purified and used as an immunogen to raise monoclonal antibodies to the subunits. These antibodies can be labelled and used in standard immunoassays to monitor the level of LFA-1, Mac-1, or p150,95 in white blood cells, and in the serum or other body fluids of patients having medical disorders associated with too many or too few cells having on their surfaces LFA-1 or related proteins. For example, diseases, e.g., AIDS, characterized by immunosuppression can be expected to be accompanied by abnormally low levels of such cells, which are instrumental in fighting infections, and such diseases can thus be monitored by monitoring levels of these proteins. Also, other disease states, e.g., autoimmune disease, allograft rejection, and graft-versus-host disease, can be expected to be characterized by abnormally high levels of such cells, and thus can also be monitored by monitoring levels of these proteins. They can also be used to diagnose leukocyte adhesion deficiency, an inherited disorder caused by lack of LFA-1, Mac-1, and p150,95 glycoproteins. Antibodies to the ß-subunit can also be

used to purify LFA-1 or related proteins by conventional immunoaffinity purification methods.

The purified proteins, particularly LFA-1, Mac-1 and/or p150,95, whether native or recombinant, can also be used therapeutically. The proteins can be administered to patients in need of such treatment in an effective amount (e.g., from 20-500 mg per kg body weight), and mixed with a pharmaceutically acceptable carrier substance such as saline. Therapeutic utility of these proteins is based on the fact that disease states such as autoimmune diseases, allograft rejections, and graft-versus-host diseases involve abnormally high levels of cell-to-cell contact mediated by the recognition and binding of LFA-1 and related proteins to target antigen presenting cells, endothelial cells, and other types of cells. The administration of LFA-1 or a related protein, or fragments thereof, will compete for receptors for the cell-bound protein, inhibiting cell-to-cell binding and thus bringing about the desired immunosuppression. A particular disease for which these proteins will be useful is the autoimmune disease rheumatoid arthritis. Preferably administration is intravenous at about 20-500 mg per kg body weight, or directly at an inflamed joint of a patient suffering from rheumatoid arthritis. Alternatively, oral administration or local application can be used by providing tablets, capsules, or solutions, or by applying lotions as required. The amount and method of administration will vary dependent upon the age and weight of the patient, and the disease to be treated. Other autoimmune diseases which can be treated include systemic lupus erythematosis, juvenile onset diabetes, multiple sclerosis, allergic conditions, eczema, ulcerative colitis, inflammatory bowel disease, Crohn's

disease, as well as allograft rejections (e.g., rejection of a transplanted kidney or heart). LFA-1, Mac-1, and p150,95 noramlly act in situ by binding to endothelial and other cells. Thus, the free proteins or peptides, which are administered, will be able to inhibit leukocyte immune responses and migration to inflammatory sites.

The ß subunit cDNA clone can be used in prenatal diagnosis of leukocyte adhesion deficiency (LAD). LAD disease is a deficiency in cell surface expression of LFA-1, Mac-1, and p150,95 and is due at least in part to a primary genetic lesion in the ß subunit. Patients with the severe form of LAD disease suffer from recurrent bacterial infections and rarely survive beyond childhood. The defect can be detected early in pregnancy since it is associated with a unique restriction fragment length polymorphism. PstI digestion of human DNA and hybridization with the 1.8 kb EcoRI fragment (shown in Fig. 2) of the ß subunit cDNA defines a restriction fragment length polymorphism (RFLP). Diagnosis of this disease is therefore performed by standard procedure using the whole or a part of this EcoRI fragment. The genomic DNAs of the parents of the fetus, and the fetus are screened with this probe and an analysis of their RFLPs made. In this way the probability that the fetus has the disease can be estimated.

Other embodiments are within the following claims.

Claims

1. A cDNA sequence encoding a) the ß-subunit of a human glycoprotein concerned with cellular adhesion, b) a biologically active fraction of said glycoprotein, c) an analog of said glycoprotein, or d) a fragment of said glycoprotein comprising at least 10% of a contiguous sequence of said cDNA.

2. A vector comprising a DNA sequence encoding the ß-subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, or an analog thereof, or a fragment thereof encoding at least 10% of a contiguous sequence of said ß-subunit.

3. Substantially pure recombinant ß-subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, or an analog thereof, or a fragment thereof comprising at least 10% of a contiguous sequence of said subunit.

4. A monoclonal antibody raised against recombinant ß-subunit of a human glycoprotein concerned with cellular adhesion, or a biologically active fraction thereof, or an analog thereof, or a fragment thereof comprising at least 10% of a contiguous sequence of said subunit.

5. The cDNA of claim 1 wherein said glycoprotein is LFA-1, Mac-1 or p150,95.

6. The vector of claim 2 wherein said glycoprotein is LFA-1, Mac-1 or p150,95, and said fragment comprises at least 80% of a contiguous sequence of said DNA.

7. The ß-subunit of claim 3 wherein said glycoprotein is LFA-1, Mac-1, or p150,95, and said fragment comprises at least 80% of a contiguous sequence of said DNA.

8. The antibody of claim 4 wherein said glycoprotein is LFA-1, Mac-1, or p150,95, and said fragment comprises at least 80% of a contiguous sequence of said DNA.

9. The cDNA sequence of claim 1, said sequence being substantially the same as at least 10% of the DNA sequence shown in Figure 1.

10. A method of treating an animal suffering from a medical condition characterized by an undesirably high level of leukocyte interaction with other cells, comprising administering to said patient an amount of LFA-1, Mac-1, or p150,95, or an effective fragment thereof, wherein said amount is effective to minimize said leukocyte interaction.

11. A method of monitoring the level of glycoproteins in an animal comprising assaying a body fluid of said patient for LFA-1, Mac-1, or p150,95, wherein said assaying comprises detecting said LFA-1, Mac-1 or p150,95 with antibodies produced to recombinant said glycoproteins.

12. A recombinant vector comprising at least a contiguous 10% section of the DNA sequence shown in Fig. 1.

13. A method for diagnosing leukocyte adhesion deficiency comprising digesting human DNA with a restriction enzyme, probing said DNA with a probe specific for a restriction fragment length polymorphism associated with said deficiency, and observing the length of a restriction fragment hybridizing to said probe; wherein the length of said restriction fragment is diagnostic of said disease.

14. The method of claim 14 wherein said probe is an 1.8 kb EcoRI fragment encoding the ß-subunit of human LFA-1, and said restriction enzyme is Pst-I.

Fig. 1 (page 1)

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     CAGGGCAGACTGGTAGCAAA GCCCCCACGCCCAGCCAGGA GCACCGCCGPGGACTCCAGC ACACCGAGGGAC ATG CTG GGC CTG CGC CCC CCA CTG CTC GCC CTG GTG GGG CTG CTC TCC CTC
                                                                              MET Leu Gly Leu Arg Pro Pro Leu Leu Ala Leu Val Gly Leu Leu Ser Leu      17

124  GGG TGC GTC CTC TCT CAG GAG TGC ACG AAG TTC AAG GTC AGC AGC TGC CGG GAA TGC ATC GAG TCG GGG CCC GGC TGC ACC TGG TGC CAG AAG CTG AAC TTC ACA GGG
     Gly (Cys) Val Leu Ser Gln Glu (Cys) Thr Lys Phe Lys Val Ser Ser (Cys) Arg Glu (Cys) Ile Glu Ser Gly Pro Gly (Cys) Thr Trp (Cys) Gln Lys Leu Asn Phe Thr Gly      53

232  CCG GGG GAT CCT GAC TCC ATT CGC TGC GAC ACC CGG CCA CAG CTG CTC ATG AGG GGC TGT GCG GCT GAC GAC ATC ATG GAC CCC ACA AGC CTC GCT GAA ACC CAG GAA
     Pro Gly Asp Pro Asp Ser Ile Arg (Cys) Asp Thr Arg Pro Gln Leu Leu MET Arg Gly (Cys) Ala Ala Asp Asp Ile MET Asp Pro Thr Ser Leu Ala Glu Thr Gln Glu      89

340  GAC CAC AAT GGG GGC CAG AAG CAG CTG TCC CCA CAA AAA GTG ACG CTT TAC CTG CGA CCA GGC CAG GCA GCA GCG TTC AAC GTG ACC TTC CGG CGG GCC AAG GGC TAC
     Asp His Asn Gly Gly Gln Lys Gln Leu Ser Pro Gln Lys Val Thr Leu Tyr Leu Arg Pro Gly Gln Ala Ala Ala Phe Asn Val Thr Phe Arg Arg Ala Lys Gly Tyr      125

448  CCC ATC GAC CTG TAC TAT CTG ATG GAC CTC TCC TAC TCC ATG CTT GAT GAC CTC AGG AAT GTC AAG AAG CTA GGT GGC GAC CTG CTC CGG GCC CTC AAC GAG ATC ACC
     Pro Ile Asp Leu Tyr Tyr Leu MET Asp Leu Ser Tyr Ser MET Leu Asp Asp Leu Arg Asn Val Lys Lys Leu Gly Gly Asp Leu Leu Arg Ala Leu Asn Glu Ile Thr      161

556  GAG TCC GGC CGC ATT GGC TTC GGG TCC TTC GTG GAC AAG ACC GTG CTG CCG TTC GTG AAC ACG CAC CCT GAT AAG CTG CGA AAC CCA TGC CCC AAC AAG GAG AAA GAG
     Glu Ser Gly Arg Ile Gly Phe Gly Ser Phe Val Asp Lys Thr Val Leu Pro Phe Val Asn Thr His Pro Asp Lys Leu Arg Asn Pro (Cys) Pro Asn Lys Glu Lys Glu      197

664  TGC CAG CCC CCG TTT GCC TTC AGG CAC GTG CTG AAG CTG ACC AAC AAC TCC AAC CAG TTT CAG ACC GAG GTC GGG AAG CAG CTG ATT TCC GGA AAC CTG GAT GCA CCC
     (Cys) Gln Pro Pro Phe Ala Phe Arg His Val Leu Lys Leu Thr Asn Asn Ser Asn Gln Phe Gln Thr Glu Val Gly Lys Gln Leu Ile Ser Gly Asn Leu Asp Ala Pro      223
               L-56a

772  GAG GGT GGG CTG GAC GCC ATG ATG CAG GTC GCC GCC TGC CCG GAG GAA ATC GGC TGG CGC AAC GTC ACG CGG CTG CTG GTG TTT GCC ACT GAT GAC GGC TTC CAT TTC
     Glu Gly Gly Leu Asp Ala MET MET Gln Val Ala Ala (Cys) Pro Glu Glu Ile Gly Trp Arg Asn Val Thr Arg Leu Leu Val Phe Ala Thr Asp Asp Gly Phe His Phe      269
                                                                                                         H-58
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Fig. 1 (continued page 2)

880 GCG GGC GAC GGA AAG CTG GGC GCC ATC CTG ACC CCC AAC GAC GGC CGC TGT CAC CTG GAG GAC AAC TTG TAC AAG AGG AGC AAC GAA TTC GAC TAC CCA TCG GTG GGC
Ala Gly Asp Gly Lys Leu Gly Ala Ile Leu Thr Pro Asn Asp Gly Arg(Cys)His Leu Glu Asp Asn Leu Tyr Lys Arg Ser Asn Glu Phe Asp Tyr Pro Ser Val Gly 305

988 CAG CTG GCG CAC AAG CTG GCT GAA AAC AAC ATC CAG CCC ATC TTC GCG GTG ACC AGT AGG ATG GTG AAG ACC TAC GAG AAA CTC ACC GAG ATC ATC CCC AAG TCA GCC
Gln Leu Ala His Lys Leu Ala Glu Asn Asn Ile Gln Pro Ile Phe Ala Val Thr Ser Arg MET Val Lys Thr Tyr Glu Lys Leu Thr Glu Ile Ile Pro Lys Ser Ala 341
P-61

1096 GTG GGG GAG CTG TCT GAG GAC TCC AGC AAT GTG GTC CAT CTC ATT AAG AAT GCT TAC AAT AAA CTC TCC TCC AGG GTC TTC CTG GAT CAC AAC GCC CTC CCC GAC ACC
Val Gly Glu Leu Ser Glu Asp Ser Ser Asn Val Val His Leu Ile Lys Asn Ala Tyr Asn Lys Leu Ser Ser Arg Val Phe Leu Asp His Asn Ala Leu Pro Asp Thr 377
H-52 L-65

1204 CTG AAA GTC ACC TAC GAC TCC TTC TGC AGC AAT GGA GTG ACG CAC AGG AAC CAG CCC AGA GGT GAC TGT GAT GGC GTG CAG ATC AAT GTC CCG ATC ACC TTC CAG GTG
Leu Lys Val Thr Tyr Asp Ser Phe(Cys)Ser Asn Gly Val Thr His Arg Asn Gln Pro Arg Gly Asp(Cys)Asp Gly Val Gln Ile Asn Val Pro Ile Thr Phe Gln Val 413

1312 AAG GTC ACG GCC ACA GAG TGC ATC CAG GAG CAG TCG TTT GTC ATC CGG GCG CTG GGC TTC ACG GAC ATA GTG ACC GTG CAG GTT CTT CCC CAG TGT GAG TGC CGG TGC
Lys Val Thr Ala Thr Glu(Cys)Ile Gln Glu Gln Ser Phe Val Ile Arg Ala Leu Gly Phe Thr Asp Ile Val Thr Val Gln Val Leu Pro Gln(Cys)Glu(Cys)Arg(Cys) 449

1420 CGG GAC CAG AGC AGA GAC CGC AGC CTC TGC CAT GGC AAG GGC TTC TTG GAG TGC GGC ATC TGC AGG TGT GAC ACT GGC TAC ATT GGG AAA AAC TGT GAG TGC CAG ACA
Arg Asp Gln Ser Arg Asp Arg Ser Leu(Cys)His Gly Lys Gly Phe Leu Glu(Cys)Gly Ile(Cys)Arg(Cys)Asp Thr Gly Tyr Ile Gly Lys Asn(Cys)Glu(Cys)Gln Thr 485
P-20

1528 CAG GGC CGG AGC AGC CAG GAG CTG GAA GGA AGC TGC CGG AAG GAC AAC AAC TCC ATC ATC TGC TCA GGG CTG GGG GAC TGT GTC TGC GGG CAG TGC CTG TGC CAC ACC
Gln Gly Arg Ser Ser Gln Glu Leu Glu Gly Ser(Cys)Arg Lys Asp Asn Asn Ser Ile Ile(Cys)Ser Gly Leu Gly Asp(Cys)Val(Cys)Gly Gln(Cys)Leu(Cys)His Thr 521
P-18 ▲

Fig. 1 (continued page 3)

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1636  AGC GAC GTC CCC GGC AAG CTG ATA TAC GGG CAG TAC TGC GAG TGT GAC ACC ATC AAC TGT GAG CGC TAC AAC GGC CAG GTC TGC GGC GGC CCG GGG AGG GGG CTC TGC
      Ser Asp Val Pro Gly Lys Leu Ile Tyr Gly Gln Tyr(Cys)Glu(Cys)Asp Thr Ile Asn(Cys)Glu Arg Tyr Asn Gly Gln Val(Cys)Gly Gly Pro Gly Arg Gly Leu(Cys)   557
                                L-56b

1744  TTC TGC GGG AAG TGC CGC TGC CAC CCG GGC TTT GAG GGC TCA GCG TGC CAG TGC GAG AGG ACC ACT GAG GGC TGC CTG AAC CCG CGG CGT GTT GAG TGT AGT GGT CGT
      Phe(Cys)Gly Lys(Cys)Arg(Cys)His Pro Gly Phe Glu Gly Ser Ala(Cys)Gln(Cys)Glu Arg Thr Thr Glu Gly(Cys)Leu Asn Pro Arg Arg Val Glu(Cys)Ser Gly Arg   593

1852  GGC CGG TGC CGC TGC AAC GTA TGC GAG TGC CAT TCA GGC TAC CAG CTG CCT CTG TGC CAG GAG TGC CCC GGC TGC CCC TCA CCC TGT GGC AAG TAC ATC TCC TGC GCC
      Gly Arg(Cys)Arg(Cys)Asn Val(Cys)Glu(Cys)His Ser Gly Tyr Gln Leu Pro Leu(Cys)Gln Glu(Cys)Pro Gly(Cys)Pro Ser Pro(Cys)Gly Lys Tyr Ile Ser(Cys)Ala   629

1960  GAG TGC CTG AAG TTC GAA AAG GGC CCC TTT GGG AAG AAC TGC AGC GCG GCG TGT CCG GGC CTG CAG CTG TCG AAC AAC CCC GTG AAG GGC AGG ACC TGC AAG GAG AGG
      Glu(Cys)Leu Lys Phe Glu Lys Gly Pro Phe Gly Lys Asn(Cys)Ser Ala Ala(Cys)Pro Gly Leu Gln Leu Ser Asn Asn Pro Val Lys Gly Arg Thr(Cys)Lys Glu Arg   665

2068  GAC TCA GAG GGC TGC TGG GTG GCC TAC ACG CTG GAG CAG CAG GAC GGG ATG GAC CGC TAC CTC ATC TAT GTG GAT GAG AGC CGA GAG TGT GTG GCA GGC CCC AAC ATC
      Asp Ser Glu Gly(Cys)Trp Val Ala Tyr Thr Leu Glu Gln Gln Asp Gly MET Asp Arg Tyr Leu Ile Tyr Val Asp Glu Ser Arg Glu(Cys)Val Ala Gly Pro Asn Ile   701

2176  GCC GCC ATC GTC GGG GGC ACC GTG GCA GGC ATC GTG CTG ATC GGC ATT CTC CTG CTG GTC ATC TGG AAG GCT CTG ATC CAC CTG AGC GAC CTC CGG GAG TAC AGG CGC
      Ala Ala Ile Val Gly Gly Thr Val Ala Gly Ile Val Leu Ile Gly Ile Leu Leu Leu Val Ile Trp Lys Ala Leu Ile His Leu Ser Asp Leu Arg Glu Tyr Arg Arg   737

2284  TTT GAG AAG GAG AAG CTC AAG TCC CAG TGG AAC AAT GAT AAT CCC CTT TTC AAG AGC GCC ACC ACG ACG GTC ATG AAC CCC AAG TTT GCT GAG AGT TAG GAGCACTTGGT
      Phe Glu Lys Glu Lys Leu Lys Ser Gln Trp Asn Asn Asp Asn Pro Leu Phe Lys Ser Ala Thr Thr Thr Val MET Asn Pro Lys Phe Ala Glu Ser   769

2403  GAAGACAAGGCCGTCAGGACCCACCATGTCTGCCCCATCACGCGGCCGAGACATGGCTTGGCCACAGCTCTTGAGGATGTCACCAATTAACCAGAAATCCAGTTATTTTCCGCCCTCAAAATGACAGCCATGGCCGGCCGGTG

2546  CTTCTGGGGGCTCGTCGGGGGGACAGCTCCACTCTGACTGGCACAGTCTTTGCATGGAGACTTGAGGAGGGCTTGAGGTTGGTGAGGTTAGGTGCGTGTTTCCTGTGCAAGTCAGGACATCAGTCTGATTAAAGGTGGTGCCA

2689  ATTTATTTACATTTAAACTTGTCAGGGTATAAAATGACATCCCATTAATTATATTGTTAATCAATCACGTGTATAGAAAAAAAAATAAAACTTCAAT   2776
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FIGURE 2

p150,95 β Subunit

P-61 sequence	L Y E N N I Q P I F A V T S
Deduced sequence	K L A E N N I Q P I F A V T S
P-20 sequence	(T/C) D T G Y I G K
Deduced sequence	R C D T G Y I G K
P-18 sequence	S S Q E L E G S (T/C) (R)
Deduced sequence	R S S Q E L E G S C R

Mac-1 β Subunit

M-58 sequence	L L V F A T D D G F H F
Deduced sequence	R L L V F A T D D G F H F
M-52 sequence	X A V G E L S E X (S) X N
Deduced sequence	K S A V G E L S E D S S N

LFA-1 β Subunit

L56a sequence	E C Q P P F A F R
Deduced sequence	K E C Q P P F A F R
L56b sequence	L I Y G Q Y C E (C) D T I
Deduced sequence	K L I Y G Q Y C E C D T I
L-65 sequence	V F L D H N A L P
Deduced sequence	R V F L D H N A L P

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/00611

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C07H 21/04; C12N 15/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/68,70,71,91,172.1,172.3,243,253,320;536/27 530/324,325,837; 935/9,10,11,22

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

CA FILE 1967-1988
BIOSIS FILE 1967-88

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	T.A. SPRINGER ET AL , "Sequence homology of the LFA-1 and Mac-1 leukocyte adhesion glycoproteins and unexpected relation to leukocyte interferon", Nature, Volume 314, pages 540-542, published 11 April 1985 by MacMillan Journals LTD (London, UK). See especially pages 540 and 541.	1,2,5 and 6
Y	F. SANCHEZ-MADRID ET AL , "Mapping of antigenic and functional epitopes on the -and subunits of two related mouse glycoproteins involved in cell interactions, LFA-1 and MAC-1", The Journal of Experimental Medicine, Volume 158, pages 586-602, published August 1983 by the Rockfeller University Press (New York, NY, USA). See especially pages 591-594.	1,2,5 and 6

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18 JUNE 1988	19 JUL 1988
International Searching Authority	**Signature of Authorized Officer**
ISA/US	JAMES MARTINELL

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	K. KURZINGER ET AL, "Structural homology of a macrophage differentiation antigen and an antigen involved in T-cell mediated killing", Nature, Volume 296, pages 668-671, published 15 April 1982 by MacMillan Journals LTD (London, UK). See entire document.	1,2,5 and 6

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [ ] Claim numbers , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. [X] Claim numbers 9 and 12, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

Claims 9 and 12 refer to Figure 1, which figure is not completely legible. See Article 17(2)(a)(ii) and 17 (2)(b).

3. [ ] Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [X] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1,2,5,6,9 and 12.
II. Claims 3 and 7.
III. Claims 4 and 8.
IV. Claim 10.
V. Claim 11.
VI. Claims 13 and 14.

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1,2,5,6,9 and 12

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.